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Separation of some metallochromic ligands by capillary zone electrophoresis and micellar electrokinetic capillary chromatography

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Abstract

Methods for the separation of several metallochromic ligands by capillary zone electrophoresis and micellar electrokinetic capillary chromatography were developed using an uncoated fused-silica capillary and an alkaline background electrolyte containing ethylenediaminetetraacetic acid (EDTA) and a zwitterionic additive. These additives were used to suppress sorption of the analytes on the capillary wall by interaction with sorbed metal ions present as impurities in the reagents used and also through polar interactions of the analytes. In model experiments it was shown that the addition of calcium or zinc ions to the background electrolyte reduces the electroosmotic flow, probably due to their sorption on silanol groups. They also have detrimental effects on the peak shape of most of the analytes. However, a separation of some metallochromic ligands could be achieved which involved complex equilibria with calcium or zinc added to the background electrolyte in an excess over the EDTA and with citrate added in an excess over the metals. Both methods yielded separation efficiencies up to approximately 500 000 theoretical plates but differed substantially in selectivity and ultraviolet–visible spectra of the separated ligands. Further, it was shown that analytes present as free ligands or as metal complexes that exhibit low effective mobilities and/or solubilities in the background electrolyte can be analysed after addition of sodium dodecylsulfate to the background electrolyte in a micellar separation mode.

1. Introduction

The uses of metallochromic ligands comprise a large area of analytical chemistry from complexometric titrations using metallochromic indicators [1,2] through photometric applications [1,2] to separation methods [3–13]. For many of these applications the purity of the ligand is not critical and some samples with a declared content of less than 50% may still function well, for example as indicators for complexometric titrations. Other analytical applications, such as photometric determination of metals or separation of metals using complexation with these ligands, may require the use of metallochromic ligands of higher quality [7]. In such cases, the reagents require thorough characterization and specification. The most widely used analytical methods [1,2] for the determination of the purity of metallochromic ligands are titration or photometry. However, these lack selectivity. Paper and thin layer chromatography may be used for

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the determination of impurities but are limited in their efficiency and sensitivity.

Although capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MECC) are electromigration separation techniques with a rapidly growing number of applications [4–6], they have not been applied to the separation of metallochromic ligands. Several papers describe isotachophoretic [7] or CZE–MECC [8–13] separations of metal complexes of metallochromic ligands; however, the aim of those separations was the determination of the metals rather than the ligands.

In this paper we present methods for the separation of several azo and triphenylmethane metallochromic ligands (Fig. 1) by CZE and MECC. These metallochromic ligands are non-selective reagents forming coloured complexes with a wide range of metals [2]. Once adequate analytical methods have been established for the measurement and control of the purity of these ligands, they are intended for use in the CZE separation of metals with selective and sensitive detection in the visible (VIS) range.

2. Experimental

2.1. Instrumentation

The instrument used was a Quanta 4000 (Waters, Milford, MA, USA) interfaced to a Maxima 820 data station (Waters). Separations were carried out using an AccuSep (Waters) fused-

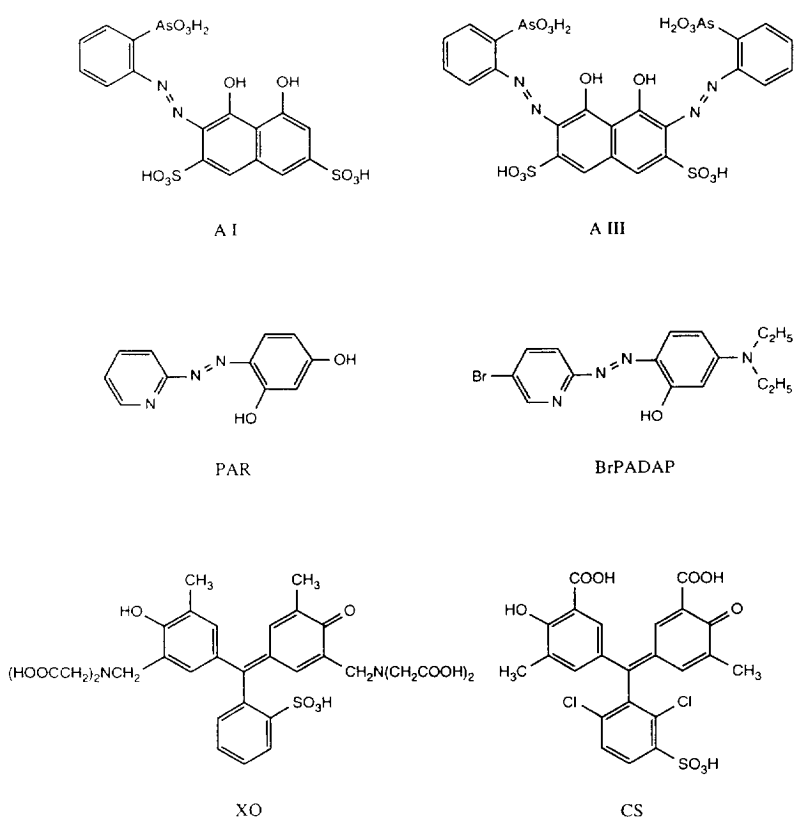

Fig. 1. Structures of metallochromic ligands: AI = arsenazo I, AIII = arsenazo III, PAR = 4-(2-pyridylazo)resorcinol, BrPADAP = 2-(5-bromo-2-pyridylazo)-5-(diethylamino)phenol, XO = xylenol Orange, CS = Chrome Azurol S (for synonyms, see Experimental).

silica capillary (60 cm × 75 mm I.D., length to detector 52 cm) initially washed with concentrated nitric acid (approximately ten capillary volumes) and then with 1 *M* sodium hydroxide overnight. An alkaline wash procedure consisting of 0.1 *M* sodium hydroxide containing 50% (v/v) methanol (solution A), followed by 1 *M* sodium hydroxide, solution A again, and finally water (at least ten capillary volumes of each) was performed whenever a background electrolyte (BGE) was changed (except for the experiments on the influence of increasing metal concentrations as given in Figs. 5 and 6). Injection was performed hydrostatically by elevating the sample at 98 mm for 10 s at the anodic side of the capillary. In order to reduce the consumption of BGE, the standard 20-ml buffer reservoir at the anodic side was modified to hold a plastic sample vial of approximately 0.6 ml, whilst at the cathodic (detector) side a buffer reservoir of 4 ml volume was used. The running voltage was +25 kV. Direct photometric detection at 254 or 546 nm was used. Separation efficiency was calculated from the peak width at half height.

2.2. Reagents and procedures

The trisodium salt of arsenazo I [2-(4,5-dihydroxy-2,7-disulfo-3-naphthylazo)phenylarsenic acid, AI], the monosodiummonohydrate salt of 4-(2-pyridylazo)resorcinol (PAR), the sodium salt of xylenol orange {3,3′-bis[N,N-di(carboxymethyl)aminomethyl)]-*o*-cresolsulfophthalein, XO} with a dye content of approximately 90% (XO sample A) and 2-(5-bromo-2-pyridylazo)-5-(diethylamino)phenol (BrPADAP) were obtained from Aldrich (Milwaukee, WI, USA). The monosodiumtrihydrate salt of arsenazo III [2,7-bis(2-arsonophenylazo)-1,8-dihydroxy-3,6-naphthalene disulfonic acid, AIII], approximately 60% content, was obtained from Sigma (St. Louis, MO, USA). The sodium salt of xylenol orange {3,3′-bis[N,N-di(carboxymethyl)aminomethyl)] - *o* - cresolsulfophthalein, XO}, purity approximately 96% (XO sample B), was obtained from BDH (Poole, UK). The trisodium salt of Chrome Azurol S (3″-sulfo-2″,6″-dichloro-3,3′-dimethyl-4-hydroxyfuchson-5,5′-dicarboxylic acid, CS) was obtained from Riedel de Haën (Hannover, Germany). Stock solutions (0.5 m*M*) of the metallochromic ligands were prepared in water (AI, AIII, PAR, XO and CS) or 50% aqueous acetonitrile (BrPADAP). Solutions of 0.1 m*M* were injected (5 s injection time) for the determination of mobilities (μ) and separation efficiencies (number of theoretical plates, *N*).

Ethylenediaminetetraacetic acid (EDTA), disodium salt, was obtained from Ajax (Sydney, Australia). Trisodium salt solutions of EDTA were prepared by titration with sodium hydroxide. Diethylenetriaminepentaacetic acid (DTPA) was obtained from Aldrich and *trans*-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid (CyDTA) from BDH. Z1-Methyl (trimethylammonium propanesulfonate) was obtained from Waters. Water was treated with a Millipore (Bedford, MA, USA) Milli-Q water purification apparatus. All other chemicals were of analytical grade.

A formate–diethanolamine (DEOLA) buffer was prepared by appropriate dilution of formic acid and adjustment to pH 9.7 with DEOLA, to give final concentrations of 20 m*M* formate and approximately 50 m*M* DEOLA. The BGEs were prepared by adding to a 10-ml volumetric flask the desired volumes of 100 m*M* EDTA, calcium diformate (prepared as a 100 m*M* solution from appropriate amounts of calcium hydroxide and formic acid) or zinc sulfate (prepared as a 100 m*M* solution in 10 m*M* sulfuric acid), 100 m*M* citric acid and Z1-Methyl. The solution was then diluted to the mark with the formate–DEOLA buffer. Before use the BGE was degassed by vacuum and filtered with a Millex-HA 0.45-mm disc filter (Millipore).

The electroosmotic flow (EOF) was determined in the non-micellar BGEs by injections of mesityloxide (MSO) added in a 10 ppm concentration to the sample. With all BGEs containing the Z1-Methyl, a positive peak (probably due to refractive index change) was observed having exactly the same migration time as MSO. Therefore, the peak caused by Z1-Methyl was used as an EOF marker.

3. Results and discussion

3.1. Separation of metallochromic ligands using BGEs containing EDTA

Preliminary experiments using AI with BGEs comprising 10 m*M* phosphate at pH 7.0, 30 m*M* borate at pH 9.5 or formate–DEOLA at pH 9.7 yielded peaks which became broader and exhibited an increased migration time after each successive injection. The alkaline wash procedure (see Experimental) restored acceptable peak shapes ($N \approx 25\,000$) for the next one or two injections, after which the peak shape started to deteriorate again.

These preliminary experiments led to the hypothesis that metal ions present as impurities in the BGEs could influence separations by binding to silanol groups on the capillary wall [14–16,24]. To prevent this, 1 m*M* EDTA was added to the BGEs. A dramatic improvement in the reproducibility of migration times and in separation efficiency resulted for all BGEs. For comparison, we also tried DTPA and found the same effect as for EDTA, and CyDTA which yielded similar results for all analytes except AI which gave a very broad peak. For all further investigations, EDTA was incorporated into the BGE.

Z1-Methyl, a zwitterionic compound (trimethylammonium propanesulfonate), was also examined as an additive to the BGE. Generally, Z1-Methyl is used to suppress polar interactions of both macromolecules [17] and small anions [18] with the capillary wall. The addition of Z1-Methyl resulted in an increase in the EOF, an overall decrease in the effective mobilities of the analytes (probably due to changes in solvation) and an additional increase in separation efficiencies relative to EDTA electrolyte (see Table 1). The formate–DEOLA buffer gave the best results in terms of reproducibility and separation efficiency (compared to borate and carbonate at the same pH). The concentration of Z1-Methyl was optimized with respect to the separation of the unknown impurity close to the AI peak (see Fig. 2a). A concentration of 0.4 *M* Z1-Methyl gave satisfactory results. Examples of separations of metallochromic ligands using this electrolyte are given in Fig. 2 (AI, AIII and CS) and Fig. 3 (XO samples A and B).

Further manipulation of the composition of the BGE showed that analytes having effective mobilities which were too low for the above electrolyte system or which precipitated from this electrolyte could be analysed after addition of sodium dodecylsulfate to the BGE with subsequent use of the MECC separation mode. This is illustrated in Fig. 4 for BrPADAP which is analysed at a pH where the molecule is uncharged. The separation efficiency was approximately 26 000 theoretical plates.

Table 1
EOF, analyte mobility and separation efficiency in background electrolytes containing EDTA

Analyte	No Z1-Methyl in BGE		0.4 *M* Z1-Methyl in BGE	
	μ (10^{9} m^2 V^{-1} s^{-1})	N ($\times 10^3$)	μ (10^{9} m^2 V^{-1} s^{-1})	N ($\times 10^3$)
EOF	+58.6	–	+69.1	–
AI	−46.8	87	−41.7	234
AIII	−43.1	154	−39.9	387
XO	−42.7	146	−38.1	368
CS	−40.8	153	−34.3	373
PAR	−22.3	287	−18.3	473

Electrolyte: 1.0 m*M* EDTA, 20 m*M* formate–DEOLA pH 9.7; for other conditions, see Experimental. AI = arsenazo I, AIII = arsenazo III, XO = xylenol Orange, CS = Chrom Azurol S, PAR = pyridylazoresorcinol.

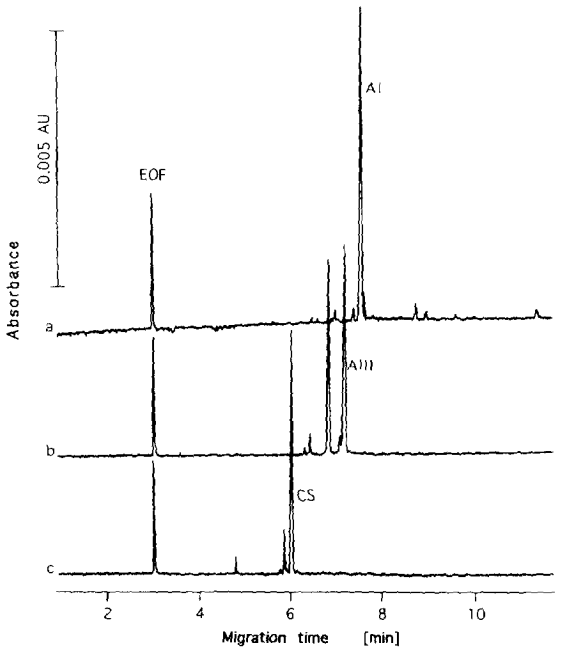

Fig. 2. Electropherograms of samples of (a) AI, (b) AIII and (c) CS in the presence of EDTA. BGE: 1.0 m*M* EDTA and 0.40 *M* Z1-Methyl in 20.0 m*M* formate–DEOLA pH 9.7. Detection wavelength: 254 nm. For other conditions, see Experimental.

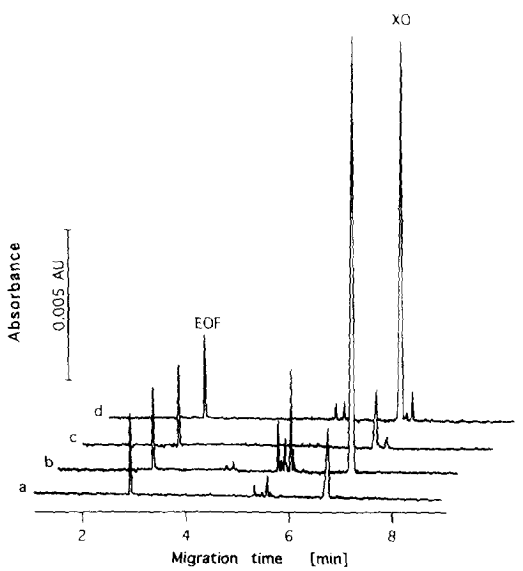

Fig. 3. Electropherograms of samples of (a, b) XO-A and (c, d) XO-B. Detection wavelength: 254 nm (a, c) or 546 nm (b, d). Other conditions as in Fig. 2.

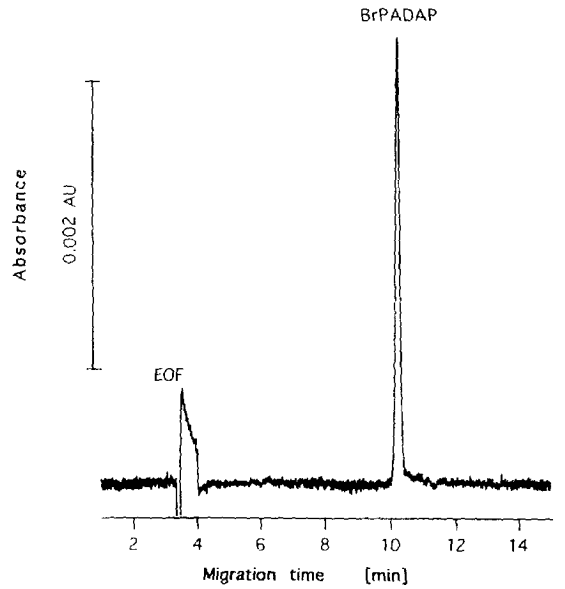

Fig. 4. Electropherogram of a sample of BrPADAP. BGE: 35 m*M* sodium dodecylsulfate, 1.0 m*M* EDTA and 0.40 *M* Z1-Methyl in 10 m*M* formate–DEOLA pH 9.7. Detection wavelength: 254 nm. For other conditions, see Experimental.

3.2. Addition of Ca(II) or Zn(II) to the BGE

The migration behaviour of the metallochromic ligands in BGEs containing metal ions was also studied. The presence of metal ions was considered to exert a possible influence on the separation efficiency and was also a potential means of manipulation of the separation selectivity of the metallochromic ligands through the involvement of these species in complexation equilibria with the metal ions added to the BGE. It has been demonstrated by Gebauer et al. [19] that a metal ion (Cd^{2+}) added to the BGE can influence effective mobilities of anions (chloride, sulfate and nitrate) through complexation and so change the selectivity of the separation. Other authors have added Zn^{2+} and Cu^{2+} to optimise the separations of oligonucleotides and peptides [20,21] or Ca^{2+} to avoid comigration of organic acids [22].

Preliminary experiments with added metal ions (Ca^{2+} as the diformate salt or Zn^{2+} as the sulfate salt) showed that without EDTA in the

BGE, added concentrations of 0.1 m*M* Ca^{2+} or Zn^{2+} ions caused the EOF to decrease and the peaks for the metallochromic ligands to exhibit severe broadening. When the BGE contained EDTA, the same effects were observed only when the metal ion concentrations in the BGE were in excess of the EDTA concentration (1.0 m*M*) (Fig. 5). Under such conditions, the observed decreased EOF was an indication of a decrease of the negative capillary wall charge, which is probably caused by sorption of the metal ions on the silanol groups of silica [14]. At the same time the effective mobilities of the ligands were decreasing and, furthermore, detrimental effects on peak shape and efficiency were observed for most analytes (except for XO and PAR in a BGE containing Ca^{2+}). In the case of PAR this latter behaviour can be explained by the fact that it does not form a complex with Ca^{2+}.

As suggested above, the results obtained in BGEs containing Ca^{2+} or Zn^{2+} ions are consistent with sorption of the metal ions on the silanol groups of the silica, causing a reduction in surface charge and hence a reduced EOF. At the same time, the free coordination sites of the sorbed metal could cause retardation of a ligand molecule through complexation effects [23]. Any such complexation–decomplexation of the solute with the metal sorbed on capillary wall would have a detrimental influence on the separation efficiency. Our results are in accordance with the recently reported [24] sorption of small aromatic acids caused by Fe^{3+} ions bound to the capillary wall as a result of Fe^{3+} ions present as trace (μg/l) impurities in the BGE.

Somewhat surprisingly, efficient separations of some metallochromic ligands could be carried out in BGEs containing Ca^{2+} or Zn^{2+} in excess over EDTA, provided that citric acid was also present at a concentration higher than the free metal concentration. Whilst such a BGE might appear unnecessarily complex, the EDTA (present as its Ca^{2+} or Zn^{2+} complex) is still a necessary component of the BGE because it can bind metal ions present as impurities that form stronger complexes than Ca^{2+} or Zn^{2+}, for example Fe^{3+}. Fig. 6 shows the dependence of

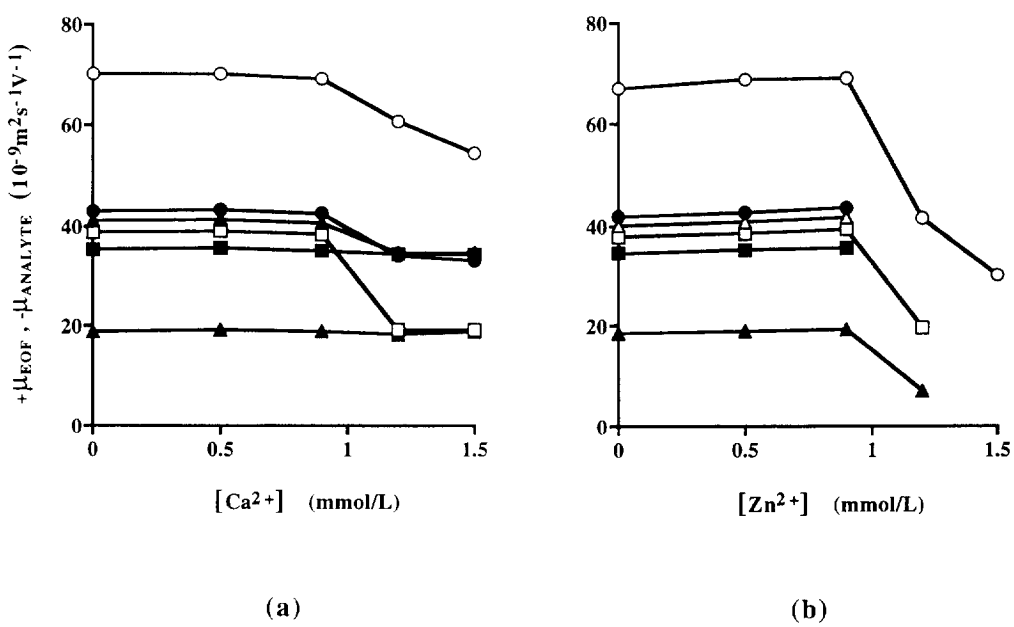

Fig. 5. Effect on EOF and analyte mobilities of (a) calcium or (b) zinc ions added to the BGE (0–1.5 m*M* Ca^{2+} or Zn^{2+}, 1.0 m*M* EDTA and 0.40 *M* Z1-Methyl in 20.0 m*M* formate–DEOLA pH 9.7): -○- = EOF, -●- = AI, -△- = AIII, -□- = XO, -■- = CS, -▲- = PAR. For other conditions, see Experimental.

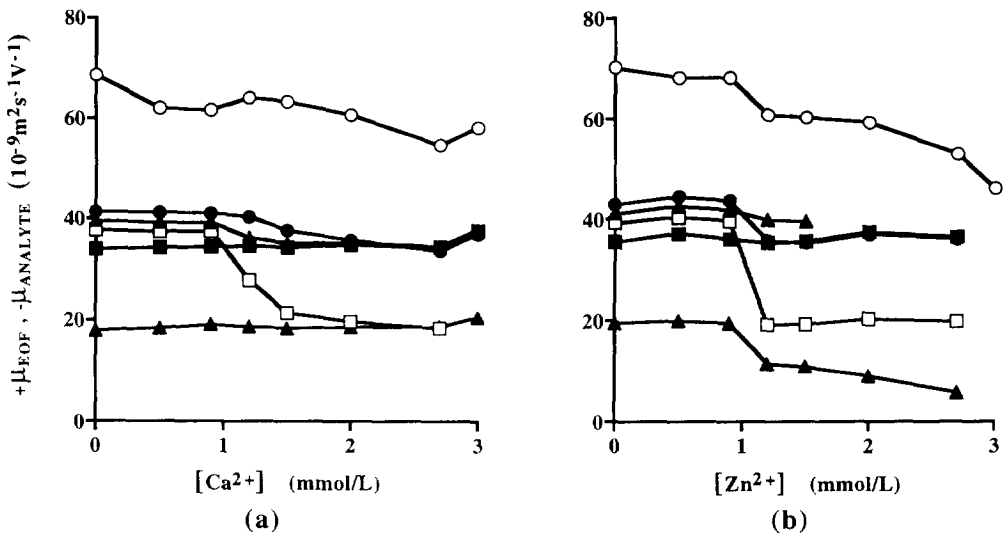

Fig. 6. Effect on EOF and analyte mobilities of (a) calcium or (b) zinc ions added to a BGE containing citrate. BGE: 0–3.0 m*M* Ca^{2+} or Zn^{2+}, 1.5 m*M* citric acid, 1.0 m*M* EDTA and 0.40 *M* Z1-Methyl in 20 m*M* formate–DEOLA at pH 9.7. Legend to curves as in Fig. 5. For other conditions, see Experimental.

the EOF and the mobilities of the metallochromic ligands on the concentration of Ca^{2+} or Zn^{2+} ions in BGEs containing citrate. Under the conditions used, the solute ligands will compete with citrate for the added metal ions, but since the citrate complexes are quite weak [25], it can be expected that the solute ligands will be the chief participators in complex formation. Visible absorption spectra of the ligands were recorded in the BGEs used in order to confirm that complex formation between the analytes and the Ca^{2+} or Zn^{2+} ions does occur. The spectra of the metallochromic ligands are known to change substantially upon complexation with metals [1,2]. The results given in Table 2 show clearly that complex formation has taken place, except for PAR with Ca^{2+} and for CS with both Ca^{2+} and Zn^{2+}.

Table 2
Wavelengths of maximum absorbance for metallochromic ligands in electrolytes without and with added metal ions

Electrolyte	Wavelengths of maximum absorbance (nm)					
	AI	AIII	XO	CS	PAR	BrPADAP
No added metal ion	500	559	579	425	414	444
1.5 m*M* Ca^{2+} added	525	597, 649	578[a]	425[b]	414[c]	525, 560[e]
1.5 m*M* Zn^{2+} added	518	587	573	425	492[d]	525, 558

Spectra measured in an electrolyte containing 1.0 m*M* EDTA, 1.5 m*M* citric acid and 0.1 *M* Z1-Methyl in 20 m*M* formate–DEOLA buffer pH 9.7. See Table 1 for solute identities.
[a] Band shape change.
[b] Increased absorbance.
[c] Decreased absorbance.
[d] Sideband ca. 520 nm.
[e] Partial complexation.

Electropherograms showing the separation of samples of XO and AI are given in Fig. 7. In addition to the good peak shapes evident from Fig. 7, a further advantage of the presence of citrate in the BGE is the fact that the decrease in the EOF at Ca^{2+} or Zn^{2+} concentrations exceeding 1.0 m*M* is considerably smaller (Fig. 6) than that without citrate (Fig. 5). Therefore, an explanation for the citrate influence on the separation may lie in the suppression of the sorption of Ca^{2+} and Zn^{2+} on the capillary wall as a result of their complexation with citrate. Alternatively, the effect might arise from saturation of the coordination sphere of the sorbed metal ions by citrate, thereby preventing sorption of the solute ligands, depending on the stability of such mixed complexes.

A 20 m*M* formate–DEOLA background electrolyte pH 9.7 containing 1.5 m*M* Zn^{2+}, 1.5 m*M* citric acid, 1.0 m*M* EDTA and 0.4 *M* Z1-Methyl could also be used for the separation of BrPADAP in a MECC mode after addition of 35 m*M* sodium dodecylsulfate. A separation efficiency of approximately 50 000 plates/m was achieved. It should be mentioned that in this MECC separation mode the citrate also prevented clouding of the BGE caused by precipitation of the Zn^{2+} salt of sodium dodecylsulfate.

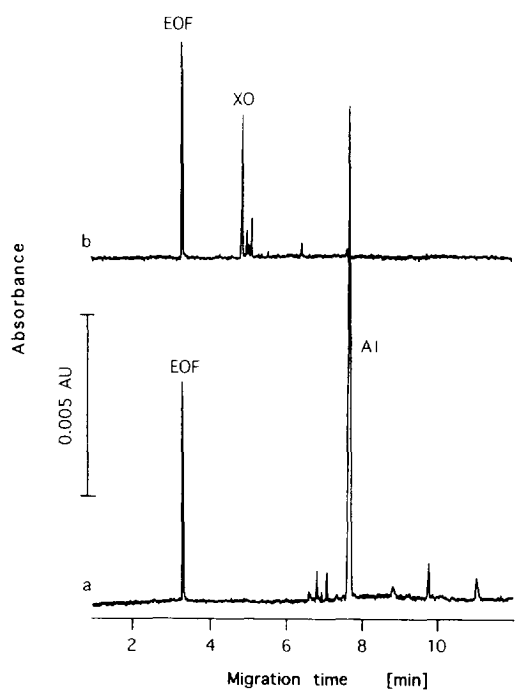

Fig. 7. Electropherograms of samples of (a) AI and (b) XO-A. BGE: 1.5 m*M* Ca(formate)$_2$, 1.5 m*M* citric acid, 1.0 m*M* EDTA and 0.40 *M* Z1-Methyl in 20 m*M* formate–DEOLA at pH 9.7. Detection wavelength: 254 nm. For other conditions, see Experimental.

4. Conclusions

CE and MECC can offer very efficient separations of metallochromic ligands if the BGE electrolyte contains a strong complexing agent to suppress the sorption of the analytes on the capillary walls via metal ions present as impurities, and Z1-Methyl to suppress other sorptions based on polar interactions. When metal ions (Ca^{2+} or Zn^{2+}) were added to the BGE in an excess over EDTA as a means of manipulating separation selectivity, decreases in the EOF and detrimental effects on the separation efficiency of most of the metallochromic ligands were observed, most probably as a result of sorptions of the metals on the capillary wall. With some ligands it was possible to retain an efficient separation even with Ca^{2+} or Zn^{2+} ions added when an excess of citrate was present in the BGE. In this way, separation selectivity could be varied if the analytes formed complexes having effective mobilities different from those of the free ligands. Furthermore, the spectra of metallochromic ligands also change substantially when complexed. This behaviour, together with the above-mentioned selectivity changes, can be used to elucidate if an unknown impurity is capable of complexation and if it has metallochromic properties.

Acknowledgement

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